

CLINICAL SCIENCE

POST-MORTEM HISTOLOGICAL PULMONARY ANALYSIS IN PATIENTS WITH HIV/AIDS

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OBJECTIVES: Certain aspects of pulmonary pathology observed in autopsies of HIV/AIDS patients are still unknown. This study considers 250 autopsies of HIV/AIDS patients who died of acute respiratory failure and describes the demographic data, etiology, and histological pulmonary findings of the various pathologies.

METHODS: The following data were obtained: age, sex, and major associated diseases (found at the autopsy). Pulmonary histopathology was categorized as: diffuse alveolar damage; pulmonary edema; alveolar hemorrhage; and acute interstitial pneumonia. Odds ratio of the HIV/AIDS-associated diseases developing a specific histopathological pattern was determined by logistic regression.

RESULTS: A total of 197 men and 53 women were studied. The mean age was 36 years. Bacterial bronchopneumonia was present in 36% (91 cases) and *Pneumocystis jirovecii* pneumonia in 27% (68) of patients. Pulmonary histopathology showed acute interstitial pneumonia in 40% (99), diffuse alveolar damage in 36% (89), pulmonary edema in 13% (33), and alveolar hemorrhage in 12% (29) of patients. Multivariate analysis showed a significant and positive association between *Pneumocystis jirovecii* pneumonia and acute interstitial pneumonia (Odds ratio, 4.51; 95% CI, 2.46 – 8.24; $p < 0.001$), severe sepsis and/or septic shock and diffuse alveolar damage (Odds ratio, 3.60; 95% CI, 1.78 – 7.27; $p < 0.001$), and cytomegalovirus and acute interstitial pneumonia (Odds ratio, 2.22; 95% CI, 1.01 – 4.93; $p = 0.05$).

CONCLUSIONS: This report is the first autopsy study to include demographic data, etiologic diagnosis, and respective histopathological findings in patients with HIV/AIDS and acute respiratory failure. Further studies are necessary to elucidate the complete pulmonary physiopathological mechanism involved with each HIV/AIDS-associated disease.

KEYWORDS: Respiratory Failure. AIDS. Pathology. Lung Autopsy. HIV.

INTRODUCTION

Millions of people worldwide are infected with the human immunodeficiency virus (HIV); moreover, acquired immunodeficiency syndrome (AIDS) has become the leading cause of death among individuals between the ages of 25 and 44 years in the United States.¹ Patients with HIV/AIDS often times have other infectious and non-infectious diseases.² Despite the use of prophylactic antibiotics over the course of infection, the lungs are the organ most frequently affected by HIV/AIDS and, hence, failure of the respiratory system

is one of the main causes of death in HIV/AIDS patients.² Little is known about the causes of death or the histological pulmonary findings in patients with HIV/AIDS at autopsy.

We performed a retrospective study of 250 autopsies on patients with HIV/AIDS whose cause of death was acute respiratory failure (ARF) in order to better describe the demographic data and etiological and histological pulmonary findings for different HIV/AIDS-associated pathologies.

MATERIAL AND METHODS

Autopsies

The present study was carried out at a tertiary complex center. From 1990 to 2000, 18,899 medical autopsies were performed. Histological pulmonary analysis was performed

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in all cases. The clinical data from patients enrolled in the study were reviewed and autopsies were performed with legal permission, after informed consent was obtained from a family member and after the approval of the Internal Review Boards. In this study, we reviewed all available microscopic tissue, macroscopic diagnosis of death at autopsy, and medical records of the patients included.

ARF was the cause of death in 3,030 (16%) patients. The diagnosis of HIV/AIDS was made in 353 (11.5%) of those patients. We excluded patients younger than one year of age, those without ARF and/or without HIV/AIDS, and 103 cases where the histological findings could not be reviewed because the pulmonary tissue was not available.

We also obtained data regarding each patient's age, sex, and major underlying associated diseases (as determined at autopsy).

After a complete review, pulmonary pathological reports were categorized as:

- Diffuse alveolar damage (DAD): diffuse involvement and uniform temporal appearance of alveolar collapse, hyaline membranes, obliterative fibrosis, neo-septa formation, and moderately organizing fibrosis
- Pulmonary edema (PE): accumulation of proteinaceous fluid in the alveolar spaces, giving the appearance of a granular, pink coagulate within such spaces
- Alveolar hemorrhage (AH): presence of blood in the alveolar spaces
- Acute interstitial pneumonia (AIP): widened and edematous alveolar septa, usually accompanied by mononuclear inflammatory infiltrate of lymphocytes, histiocytes, plasma cells, and neutrophils.

All lungs were analyzed by microscopy even when medical records indicated the patient's diagnosis. For at least four weeks, the lungs were fixed in 10% formalin prepared in 0.9% saline. We studied a minimum of five sections per lung (total ten sections per person) regardless of the presence or absence of morphologically demonstrable lesions. Paraffin-embedded tissue sections were assessed following haematoxylin and eosin staining. In order to document the presence and distribution of the wide spectrum of infectious agents to which this population is susceptible, we prepared a variety of special stains (Periodic acid-Schiff test, immunohistochemistry analysis, fluorescence, Ziehl-Neelsen, Gram, Mucicarmine, and Gomori's methenamine silver stain) for selected tissue sections. Bacterial bronchopneumonia (BBP) was defined as the presence of cell consolidation with polymorphonuclear leukocyte accumulation in bronchioles and adjacent alveoli. For the diagnosis of cytomegalovirus (CMV) and fungal pneumonia, histological evidence of lung involvement was required with or without tissue culture. Severe sepsis and/or septic shock were defined as

sepsis with the addition of organ dysfunction or clinical diagnosis of arterial hypotension, which may or may not be responsible for the aggressive fluid resuscitation. Diagnosis of *Mycobacterium tuberculosis* infection and atypical mycobacterial infection was confirmed using fluorescence and Ziehl-Neelsen techniques, and Lowenstein-Jensen culture. The proportion method and biochemistry were used for identification of all positive cultures.

Statistical analysis

Descriptive analysis of the data included median, minimum, and maximum values. The probability (odds ratio) that the major AIDS-associated diseases would develop a specific histopathological pattern was determined by logistic regression. All the statistical procedures were performed using SPSS v10.0 statistical software. Statistical significance was set at 5% (p value).

RESULTS

HIV/AIDS was described in 353 autopsies (11.65% of all patients with ARF); we analyzed 250 of these cases. The demographic data are listed in Table 1. A total of 197 (79%) HIV/AIDS-infected men and 53 (21%) HIV/AIDS-infected women were included in the study. The age at the time of death was 21 to 40 years (161 patients) for most cases. The diagnosis of HIV/AIDS showed relatively annual variation between the years 1990 and 2000.

Table 1 - Demographic analysis by sex and age in the autopsies of patients with HIV/AIDS

Age group (years)	Sex		Total
	Male	Female	
1 to 20	10	5	15 (6%)
21 to 40	126	35	161 (64%)
41 to 60	57	11	68 (27%)
>60	4	2	6 (3%)
Total	197 (79%)	53 (21%)	250

We observed a single HIV/AIDS-associated disease in 101 (40%) cases, two diseases in 67 (27%) cases, three diseases in 31 (12%) cases, and four diseases in 9 (4%) cases. No HIV/AIDS-associated diseases were detected in 40 patients (16%).

The HIV/AIDS-associated diseases in patients with ARF are shown in Table 2. BBP was present in 36% of patients (91 cases) and was the most frequent pulmonary complication found at the time of autopsy. *Pneumocystis*

Table 2 - Etiological diagnoses observed in autopsies of HIV/AIDS patients who died by ARF, along with the cause of death and main pulmonary histopathological findings

Disease	DAD	PE	AH	AIP	Total
Bacterial bronchopneumonia	31	15	17	28	91
<i>Pneumocystis jiroveci</i> pneumonia	16	2	5	45	68
Severe sepsis and/or shock septic	21	2	4	7	34
Cytomegalovirus	8	1	4	20	33
Disseminated tuberculosis	12	3	2	2	19
Toxoplasmosis	7	4	2	5	18
Pulmonary tuberculosis	8	2	3	4	17
Atypical mycobacterial infection	9	4	0	2	15
Kaposi sarcoma	3	1	3	4	11
Pulmonary embolism	5	2	0	3	10
Neurocryptococcosis	3	1	0	3	7
Non-Hodgkin's lymphoma	2	2	0	1	5
Bacterial meningitis	1	0	3	0	4
Lymphoma*	2	0	0	2	4
Histoplasmosis	3	0	0	0	3
Urinary tract infection	2	0	0	1	3
Hepatic cirrhosis*	1	1	0	1	3
Schistosomiasis	3	0	0	0	3
Acute Peritonitis*	2	0	0	1	3
Acute bacterial endocarditis	2	0	0	0	2
Pulmonary cryptococcosis	0	1	1	0	2
Neurocysticercosis	1	0	0	1	2
Chronic pneumopathy*	0	1	0	0	1
Pyelonephritis	0	0	0	1	1
Disseminated malignant neoplasm*	1	0	0	0	1
Neuropathy*	1	0	0	0	1
Duodenal ulcer	1	0	0	0	1
Acute renal failure	1	0	0	0	1
Chronic pancreatitis	0	0	0	1	1
HCB	1	0	0	0	1
Spelling	1	0	0	0	1
Venous profound thrombosis	1	0	0	0	1
Aspergillosis	0	0	1	0	1

* = No cause identified; HCB = Hepatitis virus B; DAD = Diffuse alveolar damage; PE = Pulmonary edema; AH = Alveolar hemorrhage; AIP = Acute interstitial pneumonia.

jiroveci pneumonia (PJP) was the second most frequent, observed in 27% of patients (68 cases), followed by severe sepsis and/or septic shock in 14% of patients (34 cases), CMV in 13% of patients (33 cases), disseminated tuberculosis in 8% of patients (19 cases), toxoplasmosis in 7% of patients (18 cases), and pulmonary tuberculosis in 7% of patients (17 cases).

The pulmonary histopathological analysis showed AIP in

40% of patients (99 patients), DAD in 36% of patients (89 patients), PE in 13% of patients (33 patients), and AH in 12% of patients (29 patients). The pulmonary histopathological findings observed in different HIV/AIDS-associated diseases are shown in Table 2.

Multivariate analysis (Table 3) demonstrated a statistically significant association between BBP and DAD (odds ratio [OR], 0.54; 95% confidence interval [CI], 0.31

Table 3. Multivariate analysis with the main associated diseases found in autopsies of HIV/AIDS patients, and their relationship with the respective pulmonary histopathological findings

Diseases	Pulmonary histopathological findings											
	DAD			PE			AH			AIP		
	<i>p</i> *	OR	CI 95%	<i>p</i> *	OR	CI 95%	<i>p</i> *	OR	CI 95%	<i>p</i> *	OR	CI 95%
Bacterial bronchopneumonia	0.03	0.54	0.31 - 0.94	NS	0.91	0.42 - 1.98	NS	1.85	0.78 - 4.37	0.02	0.52	0.29 - 0.91
<i>Pneumocystis jiroveci</i> pneumonia	NS	0.6	0.31 - 1.17	0.05	0.28	0.05 - 1.01	NS	0.79	0.27 - 2.33	<0.001	4.51	2.46 - 8.24
Severe sepsis and/or shock septic	<0.001	3.6	1.78 - 7.27	NS	0.38	0.08 - 1.76	NS	1.36	0.43 - 4.29	NS	0.53	0.20 - 1.36
Cytomegalovirus	NS	0.95	0.39 - 2.31	NS	0.32	0.04 - 2.51	NS	1.63	0.50 - 5.29	0.05	2.22	1.01 - 4.93
Disseminated tuberculosis	NS	2.1	0.91 - 4.87	NS	1.18	0.32 - 4.41	NS	0.94	0.20 - 4.42	0.01	0.14	0.03 - 0.63
Toxoplasmosis	NS	0.78	0.3 - 2.02	NS	1.74	0.54 - 5.56	NS	1.03	0.22 - 4.80	NS	0.42	0.14 - 1.25
Pulmonary tuberculosis	NS	1.59	0.63 - 4.03	NS	0.88	0.19 - 4.09	NS	1.37	0.36 - 5.19	NS	0.43	0.13 - 1.41
Atypical mycobacterial infection	NS	2.61	0.94 - 7.27	NS	3.18	0.89 - 11.31	NS	0.001	0 - 3.8	NS	0.33	0.07 - 1.56
Kaposi sarcoma	NS	0.74	0.20 - 2.77	NS	0.65	0.08 - 5.31	NS	2.77	0.72 - 10.72	NS	0.76	0.22 - 2.64
Pulmonary embolism	NS	1.73	0.54 - 5.52	NS	1.49	0.31 - 7.24	NS	0.001	0.001 - 6.0	NS	0.63	0.15 - 2.54

OR = Odds ratio; CI = Confidence interval; DAD = Diffuse alveolar damage; PE = Pulmonary edema; AH = Alveolar hemorrhage; AIP = Acute interstitial pneumonia; NS = Not statistically significant. **p* < 0.05

– 0.94; *p* = 0.03), BBP and AIP (OR, 0.52; 95% CI, 0.29 – 0.91; *p* = 0.02), PJP and AIP (OR, 4.51; 95% CI, 2.46 – 8.24; *p* < 0.001), severe sepsis and/or septic shock and DAD (OR, 3.60; 95% CI, 1.78 - 7.27; *p* < 0.001), CMV and AIP (OR, 2.22; 95% CI, 1.01 – 4.93; *p* = 0.05), and disseminated tuberculosis and AIP (OR, 0.14; 95% CI, 0.03 – 0.63; *p* = 0.01).

DISCUSSION

This is the first report of an autopsy study that includes the demographic data, etiologic diagnosis, and respective histopathological findings in patients with HIV/AIDS and acute respiratory failure. HIV/AIDS was described in 250 autopsies. As described, BBP was present in 36% and PJP in 27% of patients. Pulmonary histopathology showed acute interstitial pneumonia in 40%, diffuse alveolar damage in 36%, pulmonary edema in 13%, and alveolar hemorrhage in 12% of patients. Multivariate analysis showed a significant and positive association between PJP and acute interstitial pneumonia, severe sepsis and/or septic shock and diffuse alveolar damage, and finally cytomegalovirus and acute interstitial pneumonia.

The accurate diagnosis and early treatment of HIV/AIDS-associated diseases remain important problems in the management of patients with HIV/AIDS.² Pulmonary involvement has been reported in 80 – 94% of patients with HIV/AIDS-associated diseases.² Despite recent technological

advances in diagnosis, the autopsy has remained an important complementary tool for the identification and understanding of diseases in patients with HIV/AIDS.¹ Recent autopsy studies have shown important differences between autopsy findings and the clinical diagnosis *antemortem*.¹⁻³ In the present study, we observed a high prevalence (16%) of patients with HIV/AIDS who also had ARF as the cause of death. Most analyzed patients were males (79%); the mean age was 36 years. Studies in other countries have shown similar data.^{1,4} A retrospective study carried out between 1988 and 2000 considered 143 autopsies of patients with HIV/AIDS in India; this study showed a prevalence of HIV/AIDS of 72% in men and 48% in patients 21 to 30 years old.²

In the present study, the diagnosis of HIV/AIDS showed relatively little annual variation between the years 1990 and 2000. The beginning of the study was characterized by the greatest number of cases, which then progressively decreased and finally showed a small, transient increase. This variation was probably due to the decrease in the incidence of HIV/AIDS, better efficacy and distribution of antiretroviral therapy, and recent technological advances in diagnosis.

We observed a single HIV/AIDS-associated disease in 101 (40%) cases, and two or more HIV/AIDS-associated diseases in 44% of patients. In an autopsy study of patients with HIV/AIDS performed in the United States, Afessa et al. showed the presence of two or more associated diagnoses in 52% of the cases studied.¹ Hence, an important association

between different pulmonary diseases in patients with HIV/AIDS and ARF was established, which could indicate the necessity of a different therapeutic strategy for these patients.

BBP was present in 36% of the patients (91 cases) and it was the most frequent pulmonary complication found during autopsy. The importance of BBP was not fully recognized in the beginning of the HIV/AIDS epidemic. However, recent studies have shown that bacterial infections occur more frequently than other opportunistic infections in patients with HIV/AIDS.^{1,4-7} The occurrence of bacterial infections in patients with HIV/AIDS is also a reflection of their immunosuppression, and the most frequent pathogens are *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae*, and *Pseudomonas aeruginosa*.¹⁻⁵ Rana et al. analyzed autopsies in Kenya and showed an association between tuberculosis and BBP in 96% of patients with HIV/AIDS.⁶ Even in pediatrics studies, BBP appeared as the major cause of ARF in autopsies of patients with HIV/AIDS.⁵

At the onset of the HIV/AIDS epidemic, PJP was the most common pulmonary opportunistic infection in developed countries, although recent advances in early detection, primary and secondary prophylaxis, and the aggressive treatment of PJP have resulted in a decline in prevalence.² The involvement of PJP in pulmonary infection has been reported in 5 – 24% of cases.^{1-4,8-10} In the present study, the prevalence of PJP was 27%, which is different from that found in developing countries, where tuberculosis is the most prevalent opportunistic infection in patients with HIV/AIDS.^{1-4,8-10} We believe that the high prevalence of PJP in our study may be due to variable distribution in our country. Morris et al. used polymerase chain reaction (PCR) to detect the presence of *Pneumocystis* in autopsies of patients with HIV/AIDS; the rate of *Pneumocystis* colonization was 46%, although the infection levels were lower.¹¹

Several autopsy studies in patients with HIV/AIDS have reported the presence of CMV infection in 7 – 81% of patient cases.^{2,4,9,10,12-16} In the present study, CMV pneumonitis was found in 13% of patients. CMV infection has been clinically undiagnosed and, thus, it has shown an important discrepancy with autopsy results.² In a study carried out in India, Lanjewar et al. detected CMV in 7% of cases.² Ohtomo et al. conducted an autopsy study in Japan between 1986 and 1997 and found that CMV infections, present in 32% of cases, were the major opportunistic infection in patients with HIV/AIDS.⁹ Similar findings were reported by McKenzie et al., but with a higher rate of infection (81%).¹⁰

Tuberculosis was found in 15% of the cases in our study (8% as the disseminated form and 7% as the pulmonary form). To date, autopsy studies have found the presence of

Mycobacterium tuberculosis infection in 5 – 59% of cases.^(2,5,6,8,9,17-19) In developed countries such as Italy and Japan, the prevalence of tuberculosis in autopsies of patients with HIV/AIDS is 5 – 7%, whereas these rates were found to be 40 – 59% in developing countries, markedly different from the values observed in our study.^{2,5,6,8,9,17-19} One of the most common characteristics of tuberculosis is its chronic form of development and progression, which justify the lower rates observed in the present study, where only patients with ARF were studied.

The etiological diagnosis, such as atypical mycobacterial infection and Kaposi's sarcoma, also showed an important prevalence in our study. Atypical mycobacterial infection was present in 6% of cases. As with tuberculosis, we observed lower levels than those found in the literature (15 – 33%), probably due to the fact that only patients with ARF were studied.^{1,4,10,17} Kaposi's sarcoma, present in 4.5% of cases, was the most common malignancy affecting patients with HIV/AIDS. A similar prevalence (1 – 11%) has been reported in other autopsy studies of patients with HIV/AIDS.^{2,5,8-10,13,16}

Based upon pulmonary histopathological analysis, AIP was the most common pattern observed (40% of cases), followed by DAD (36% of cases). No similar data were found in the literature regarding the autopsies of patients with HIV/AIDS. However, these findings are in agreement with the higher prevalence of opportunistic infections, mainly viral, fungal, and mycobacterial infections, which frequently cause the development of AIP. Other contributing factors include the impeded mechanical ventilation, which could accelerate the development of these histopathologies. The microscopic analysis showed that the alveolar septa were widened and edematous; furthermore, they presented with a mononuclear inflammatory infiltrate of lymphocytes, histiocytes, plasma cells, and neutrophils. The association between PJC and CMV with AIP was also observed. Notably, a negative association was observed between BBP and DAD, disseminated tuberculosis and AIP, and also BBP and AIP. We had expected a positive association between these factors, but we observed that the acute inflammatory pattern was extensively modified, probably due to immunosuppression. Some studies have proposed the presence of cell atypias, immunosuppression, antiretroviral therapy, antibiotics, and opportunistic infections as the main modifiers of the inflammatory response in patients with HIV/AIDS and bacterial infections.^{20,21}

CONCLUSIONS

Despite recent technological advances in diagnosis, the autopsy has remained an important complementary tool for

the identification and understanding of diseases in patients with HIV/AIDS. The lungs are the most commonly affected organ in patients with HIV/AIDS. BBP was the most common diagnosis, followed by PJC. The most prevalent pulmonary histopathological pattern was AIP, which

suggested a positive association between PJC and CMV.

Further studies are necessary to elucidate the complete pulmonary physiopathological mechanism involved with each AIDS-associated disease.

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